Amendments to the Specification:

Please amend the paragraph on page 1, lines 6-7, and its header, as follows:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a U.S. national stage entry under 35 U.S.C. §371 of PCT International Patent Application No. PCT/US2005/004410, filed February 14, 2005, and claims priority to ~~This is a U.S. National Phase of PCT Application No. PCT/US2005/004410, filed December 16, 2004, which claims the benefit of~~ U.S. Provisional Application No. 60/544,376, filed February 13, 2004.

Please amend the paragraph on page 1, lines 10-12, and its header, as follows:

~~STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT~~

~~The U. S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of CA095019 and GM38511, awarded by The National Institutes of Health.~~

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under grant numbers CA095019 and GM38511 awarded by the National Institutes of Health. The government has certain rights in the invention.

For the following amendments, please note that the headers for paragraphs were underlined in the original text and do not indicate a change made herein.

Please amend the paragraph beginning on page 26, line 21 as follows.

Materials and Chemicals were obtained from Aldrich, except for piperidine, protected amino acids, 1-hydroxybenzotriazole (HOBt), benzotriazole-1-yloxytris-pyrrolidinophosphonium hexafluorophosphate (PyBOP), N,N,N',N'-tetramethyl-(succinimido)uranium tetrafluoroborate (TSTU), and TentaGel resin, which were obtained from Advanced Chemtech and Bachem. PKC enzymes were purchased from PanVera. Unifilter plates were obtained from Whatman. Solvent-resistant MULTISCREEN® ~~MultiScreen~~ 96-well filter plates and the MULTISCREEN® ~~Multiscreen~~ 96-well filterplate vacuum manifold were purchased from Millipore Corp.

Please amend the paragraph beginning on page 29, line 1 as follows.

Protein Kinase Cα Assay (library screening). 20 µL of 37.5 µM peptide inhibitor candidate (from each well of libraries **I**, **II**, **III**, and **IV**) was added to each well of 96 multiwell assay plates containing 20 µL assay buffer [62.5 mM HEPES (pH 7.5), 50 µM Ac-Ser-Phe-Arg-Arg-Arg-NH_2 (SEQ ID NO:11), 2.0 mM $CaCl_2 \cdot 2H_2O$, 34 mM $MgCl_2 \cdot 6H_2O$, 1.4 mM EGTA·Na, phosphatidylserine (225 µg/mL), diacylglycerol (40 µg/mL) and 313 µM cold ATP supplemented with 70 - 163 µCi/well [γ-^{33}P]ATP for radioactive detection]. 10 µL enzyme diluted buffer containing 20 mM Tris (pH 7.5), PKC (0.5 ng/µL), 1 mM DTT, BSA (730 µg/mL) and 1 mM EDTA·4Na·$2H_2O$ were added last to initiate the reaction. Total reaction volume was 50 µL. After a 10-min incubation at 30 ˚C, 100 µL of 6% phosphoric acid was added to each well to stop the reaction (total volume: 150 µL). Following an additional 5 min incubation at ambient temperature, 75 µL from each reaction well was transferred into each well of a Unifilter

(P81 cellulose phosphate paper) assay plate and washed four times with 0.1% phosphoric acid in water. Scintillation solution was added to each well and ^{33}P-incorporation measured by scintillation counting with a MICROBETA® ~~MicroBeta™~~ TriLux & MicroBeta JET (Perkin Elmer). IC_{50} values were calculated using GraFit (Erithacus Software Limited) and K_i values were calculated using Enzyme Kinetics, SigmaPlot (SPSS Inc.)

Please amend the paragraphs beginning on page 30, lines 8 and 20 as follows.

Protein Kinase Cα Assay (K_i determination for peptide 6 versus variable Ac-Ser-Phe-Arg-Arg-Arg-NH_2 substrate) (SEQ ID NO:11). The assay was conducted as described above for peptide **3** versus variable peptide substrate with the exception that the enzyme solution contained a ten-fold lower concentration of PKCα (0.05 ng/μL). The reaction was initiated as described above. After an 18-min incubation at 30 ˚C, 100 μL of 6% phosphoric acid was added to each well to stop the reaction (total volume: 150 μL). Following an additional 5 min incubation at ambient temperature, 75 μL from each reaction well was transferred into each well of a Unifilter (P81 cellulose phosphate paper) assay plate and washed four times with 0.1% phosphoric acid in water. Scintillation solution was added to each well and ^{33}P-incorporation measured by scintillation counting with a MICROBETA® ~~MicroBeta™~~ TriLux & MicroBeta JET (Perkin Elmer). IC_{50} values were calculated using GraFit (Erithacus Software Limited) and K_i values were calculated using Enzyme Kinetics, SigmaPlot (SPSS Inc.)

Protein Kinase Cα Assay (IC_{50} determination for peptide 6 versus histone III-S substrate). 20 μL assay buffer solution containing 62.5 mM Hepes (pH 7.5), $CaCl_2.2H_2O$ (1.88 mM), $MgCl_2.6H_2O$ (31.3 mM), EGTA.Na (1.3 mM), PS (450 μg/mL), DAG 40 μg/mL, cold ATP (313 μM), supplemented with 70-163 μCi/well [^{33}P]ATP for radioactive detection with 625 nM histone III-S as substrate were added to 20 μL of a

solution containing peptide **6** at various concentrations (4, 8, 16, 32, 64, 128, 256, 512 nM). 10 µL enzyme buffer solution containing 20 mM Tris (pH 7.5), PKCα (0.05 ng/µL), 1 mM DTT, BSA (730 µg/ mL), and EDTA.4Na.2H_2O (1 mM) were added to start the reaction. After an 18 min incubation at 30 ˚C, 100 µL of 6% phosphoric acid was added to quench the reaction at room temperature. The resulting volume in each individual well is 150 µL. Following an additional 5 min incubation, 75 µL from each well was transferred to Unifilter P81 cellulose phosphate paper and washed with 0.1% phosphoric acid (3 x 200 µL) and water (200 µL). Scintillation solution was added to each well and ^{33}P incorporation measured by scintillation counting with MICROBETA® ~~MicroBeta~~™ TriLux & MicroBeta JET (Perkin Elmer). The IC_{50} value for compound **6** as an inhibitor of histone III-S phosphorylation was found to be 31.7 ± 0.8 nM as calculated using GraFit (Erithacus Software Limited).

Please amend the paragraph beginning on page 34, line 27 as follows.

The PKC isoforms employed in this study were purchased from Panvera. Radioactive γ-P^{33}-ATP was obtained from AmerSham Biosciences. 96-well (2 mL/well) Uniplates and P81 Cellulose Phosphate Paper Unifilter Plates were obtained from Whatman Inc. Solvent-resistant MULTISCREEN® ~~MultiScreen~~ 96-well (300 µL/well) filter plates, the MULTISCREEN® ~~Multiscreen~~ Resist Vacuum Manifold, and Tape Multiscreen Harvester CL Plates were purchased from Millipore Corporation. Radioactive intensity of the library assays and IC_{50} determinations was detected by 1450 Microbeta liquid scintillation counter. GraFit Version 5 was used to determine the IC_{50} values.

Please amend the paragraph beginning on page 35, line 34 as follows.

Library I. The Fmoc group in consensus peptide-1 was removed with 20% piperidine in DMF and the resin was subsequently mixed with a solution of Ac_2O (0.51 g, 5 mmol) in DMF (40 mL). Then 1 g (10 mmol) of NMM was added and the mixture was shaken for 1 h. The resin was washed with DMF (3 x 20 mL), isopropyl alcohol (3 x 20 mL), and CH_2Cl_2 (3 x 20 mL) and subsequently dried *in vacuo*. The Adpoc group was selectively removed by adding the resin to a 40 mL solution of 3% TFA in CH_2Cl_2. The mixture was shaken for 5 min, the TFA solution drained, and the resin washed with DMF (3 x 20 mL). Exposure to TFA followed by washing with DMF was repeated two additional times. The resin was subsequently dried *in vacuo*. The peptide–bound resin was distributed in 5 mg quantities into individual wells of solvent-resistant MULTISCREEN® ~~MultiScreen™~~ 96-well filter plates (8 plates total). To each well was added a solution of a carboxylic acid (200 eq.) in 100 µL DMF and a second solution containing PyBOP (200 eq.), HOBt (200 eq.), and NMM (400 eq.) in 100 µL of DMF. A total of 720 different carboxylic acids were employed. The plates were gently shaken overnight, and then each well subjected to a series of washing steps (3 x 200 µL of DMF, 3 x 200 µL of isopropyl alcohol, and 3 x 200 µL of CH_2Cl_2). All the side chain protecting groups, Boc, Trt, and Pbf, were removed via treatment with TFA:H_2O:TIS (95:2.5:2.5) for 2 h at ambient temperature. The resin was washed with DMF (3 x 20 mL), isopropyl alcohol (3 x 20 mL), and CH_2Cl_2 (3 x 20 mL) and the peptide-nonpeptide conjugates subsequently cleaved from the disulfide-containing resin with 10 mM dithiothreitol (DTT) in 50 mM Tris, pH 7.5 (1 x 200 µL for 3 h and 2x 150 µL for 3 h each) and filtered into a receiving set of 96-well plates using a vacuum manifold (final volume of 500 µL). The coupling efficiency of the acylation reaction and the purity of peptide-nonpeptide conjugates were assessed via the ninhydrin test and RP-HPLC, respectively. No free *N*-terminal peptide was detected, and >90% of total ligand was cleaved from the resin with the first DTT cleaving step. The final two DTT washings

removed the residual resin-bound peptide. Compound purity was >90% as assessed by HPLC, and the HPLC-purified compounds (i.e. removal of Tris buffer and DTT) were characterized by MALDI-MS. These peptides, containing 720 different groups at the Dap ß-amino position in 8 plates, comprise Library I.

Please amend the paragraph beginning on page 36, line 25 as follows.

Library II. The Adpoc group in consensus peptide-1 (5 g resin) was selectively removed with 40 mL of 3% TFA in CH_2Cl_2 (3 x 5 min) and the resulting free amine on the side chain of the Dap residue was coupled with 0.95 g (5 mmol) of 10-hydroxydecanoic acid in the presence of 3.25 g (5 mmol) of PyBop, 0.77 g (5 mmol) of HOBt, and 1.01 g (10 mmol) of NMM in 40 mL of DMF. The reaction mixture was shaken overnight. The solvent was removed from the resin and the resin subsequently washed with DMF (3 x 20 mL), isopropyl alcohol (3 x 20 mL), and CH_2Cl_2 (3 x 20 mL). The resin was exposed to 40 mL of 20% piperidine solution in DMF (2 x 20 min). The Fmoc group at the N-terminus was removed, the resin washed, dried, and then added in 5 mg quantities to the individual wells of 8 solvent-resistant MULTISCREEN® ~~MultiScreen™~~ 96-well filter plates. The following procedures, as described for Library I, were employed: the resin in each well was coupled with one of 720 different carboxylic acids, the side chain protecting groups were removed, and the peptides were cleaved from the resin to furnish Library II.

Please amend the paragraphs beginning on page 37, lines 3 and 17 as follows.

Library III. The Adpoc group in consensus peptide-1 (5 g resin) was selectively removed with 40 mL of 3% TFA in CH_2Cl_2 (3 x 5 min) and the resulting free amine on the side chain of the Dap residue was coupled with 1.12 g (5 mmol) of 4-nitrohippuric acid in the presence of 3.25 g (5 mmol) of PyBop, 0.77 g (5 mmol) of HOBt, and 1.01 g

(10 mmol) of NMM in 40 mL of DMF. The reaction mixture was shaken overnight. The solvent was removed from the resin and the resin subsequently washed with DMF (3 x 20 mL), isopropyl alcohol (3 x 20 mL), and CH_2Cl_2 (3 x 20 mL). The resin was exposed to 40 mL of 20% piperidine solution in DMF (2 x 20 min). The Fmoc group at the N-terminus was removed, the resin washed and dried. The peptide-resin at this stage is H_2N-Arg(Pbf)-Arg(Pbf)-Gln(Trt)-Dap[3-NH-(4-nitrohippuric-yl)]-Phe-Met-Tyr(t-butyl)-Phe-S-S-Tentagel-Resin ("consensus peptide-2"). The peptide-resin was added in 5 mg quantities to the individual wells of 8 solvent-resistant MULTISCREEN® ~~MultiScreen™~~ 96-well filter plates. The following procedures, as described for Library I, were employed: the resin in each well was coupled with one of 720 different carboxylic acids, the side chain protecting groups were removed, and the peptides were cleaved from the resin to furnish Library III.

Library IV. 1.1 g of resin consensus peptide-2 was distributed in 10 mg quantities into 54 wells of a solvent-resistant MULTISCREEN® ~~MultiScreen™~~ 96-well filter plate. 100 µL of a solution of an aromatic aldehyde in DMSO (0.5 M, 0.05 mmol, 50 eq.) was added to each well. 180 mg (3 mmol) $NaCNBH_3$ was dissolved in 6 mL of trimethyl orthoformate (TMOF) with shaking for 5 min, and the resultant solution was added in 100 µL portions to each well. The plate was sealed and shaken for 6 h. The solvent was then drained and the resin washed successively with DMF (3 x 100 µL), DMF/H_2O (1/1, 3 x 100 µL), H_2O (3 x 100 µL), MeOH/CH_2Cl_2 (1/1, 3 x 100 µL), and CH_2Cl_2 (3 x 100 µL). The resin was then exposed to a second round of reductive alkylation and washing steps. All the side chain protecting groups, Boc, Trt and Pbf, were removed with TFA:H_2O:TIS (95:2.5:2.5) in 2 h at ambient temperature. The peptide-nonpeptide conjugates were cleaved from the disulfide-containing resin with 10 mM DTT in 50 mM Tris, pH 7.5 (1 x 200 µL for 3 h and 2 x 150 µL for 3 h each) and

filtered into a receiving set of 96-well plate using the vacuum manifold (final volume of 500 µL). These peptides in 54 wells comprise Library IV.

Please amend the paragraph beginning on page 41, line 28 as follows.

Protein kinase C δ screen. 20 µL of 12.5 µM peptide inhibitor candidate (from each well of libraries **I**, **II**, and **III**) was added to individual wells of 96 multiwell assay plates containing 20 µL assay buffer [62.5 mM HEPES (pH 7.5), 50 µM substrate, 30 mM $MgCl_2 \cdot 6H_2O$, 1.0 mM EGTA·Na, PS (50 µg/mL), DAG (10 µg/mL) and 300 µM cold ATP supplemented with 55 µCi/96-well plate (0.5 µCi/well) [γ-^{33}P] ATP for radioactive detection. 10 µL of an enzyme buffer solution, containing 20 mM Tris (pH 7.5), PKCδ (10 ng/well), 0.5 mM DTT, 0.375 mg/mL BSA, and 0.5 mM EDTA·4Na·$2H_2O$, was added to initiate the reaction. Total reaction volume in each well was 50 µL. After a 10-min incubation at 30 ˚C, 100 µL of 6% phosphoric acid was added to each well to stop the reaction (total volume: 150 µL). Following an additional 5 min incubation at ambient temperature, 75 µL from each reaction well was transferred into each well of a Unifilter (P81 cellulose phosphate paper) assay plate and washed four times with 0.1% phosphoric acid in water. ScintiSafe 30% solution was added to each well and ^{33}P-incorporation measured by scintillation counting with a 1420 MICROBETA® ~~MicroBeta~~™ TriLux & MicroBeta JET (Perkin Elmer). Lead compounds from the library were combined into a single 96 well plate and re-assayed as described above in order to identify the best inhibitor.

Please amend the paragraph beginning on page 42, line 8 as follows.

Protein Kinase C ζ screen. 20 µL of 12.5 µM peptide inhibitor candidate (from each well of libraries **I**, **II**, and **III**) was added to individual wells of 96 multiwell assay plates containing 20 µL assay buffer [62.5 mM HEPES (pH 7.5), 50 µM substrate, 30

mM $MgCl_2 \cdot 6H_2O$, 1.0 mM EGTA·Na, PS (50 µg/mL), and 300 µM cold ATP supplemented with 75 µCi/96-well plate (0.75 µCi/well) [γ-^{33}P] ATP for radioactive detection. 10 µL of an enzyme buffer solution, containing 20 mM Tris (pH 7.5), PKC ζ (10 ng/well), 0.5 mM DTT, 0.375 mg/mL BSA, and 0.5 mM EDTA·4Na·$2H_2O$, was added at last to initiate the reaction. Total reaction volume in each well was 50 µL. After a 10-min incubation at 30 ˚C, 100 µL of 6% phosphoric acid was added to each well to quench the reaction (total volume: 150 µL). Following an additional 5 min incubation at ambient temperature, 75 µL from each reaction well was transferred into each well of a Unifilter (P81 cellulose phosphate paper) assay plate and washed four times with 0.1% phosphoric acid in water. ScintiSafe 30% solution was added to each well and ^{33}P-incorporation measured by scintillation counting with a 1420 MICROBETA® ~~MicroBeta™~~ TriLux & MicroBeta JET (Perkin Elmer). Lead compounds from the library were combined into a single 96 well plate and re-assayed as described above in order to identify the best inhibitor.

Please amend the paragraph beginning on page 44, line 3 as follows.

As a starting point, we employed a slightly modified and amalgamated version of the previously described consensus active site recognition sequences (Nishikawa et al., 1997) of PKC δ and ζ: Arg-Arg-Gln-Gly-Dap-Phe-Met-Tyr-Phe [where Dap = (L)-2,3-diaminopropionic acid] (SEQ ID NO:17). In general, peptides containing consensus sequences bind modestly, at best, to their intended protein targets. Indeed, the simple diacetylated consensus derivative **A** exhibits an IC_{50} of 50 µM for PKC δ and 80 µM for PKC ζ. We reasoned that there is a good likelihood that unnatural substituents, positioned off the consensus sequence scaffold, might engage in high affinity interactions with subsites that lie adjacent to the active site region. In order to explore this notion, the consensus peptide **1** on the disulfide-linked Tentagel resin was prepared

as shown in FIG. 6. Following solid phase peptide synthesis, the amine side chain of the Dap residue was deprotected and the peptide-S-S-resin subsequently added to individual wells of solvent-resistant MULTISCREEN® ~~MultiScreen™~~ 96-well filter plates. Each well contained 1 of 720 different, structurally diverse (hydrophobic, hydrophilic, cyclic, acyclic, charged, uncharged, etc), commercially available carboxylic acids. Condensation of the Dap amine side chain with the various acids furnished the amides **2** (FIG. 7). In addition, the corresponding free amine (non-acylated) derivative **1** was included in the peptide library. The entire synthetic strategy is easily automated using a liquid handling robot. The peptides were side chain-deprotected, released from the resin with assay buffer (which contains DTT), and collected in an assay-ready form for subsequent screening [compounds **3** ("Library I")]. The latter was performed in a 96 well plate format versus PKC ζ. Two lead inhibitors were identified, designated as compounds **B** (IC_{50} = 0.85 ± 0.2 μM) and **C** (IC_{50} = 6.4 ± 0.3 μM) (FIG. 7). The IC_{50} value of the former is approximately 100-fold better than that of the diacetylated parent peptide **A**. However, neither **B** nor **C** displays significant (<10-fold) selectivity for PKC ζ versus a subset of other PKCs (data not shown).